

We claim:

- 5 1. An immunoassay system for determining the presence or amount of a troponin form or a group of troponin forms in a whole blood, plasma or serum sample suspected of containing troponin from damaged heart muscle, said system comprising:
- 10 a) formation of an antibody conjugate comprising an antibody coupled to a signal generating element, said antibody capable of specifically binding to cardiac specific regions of a form of troponin or a group of troponin forms;
- 15 b) formation of a reaction mixture comprising said whole blood, plasma or serum sample incubated with said antibody conjugate;
- 20 c) application of said reaction mixture to a surface to which is bound at least one capture antibody capable of specifically binding to cardiac specific regions of a form of troponin or a group of troponin forms in said antibody conjugate, said capture antibody binding said antibody conjugate, whereby the immobilized conjugate produces a detectable signal upon formation of sandwich complexes; and,
- 25 d) relation of detectable signal to the presence or amount of said troponin form or said group of troponin forms in said sample.
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2. The immunoassay system of claim 1 wherein the conjugate antibody of step a) is a sensitive antibody which comprises the ability to specifically bind a single troponin form; and step d) comprises relation of detectable signal to the presence or amount of said single troponin form in said sample.

3. The immunoassay system of claim 1 wherein the capture antibody of step c) is a sensitive antibody which comprises the ability to specifically bind a single troponin form; and step d) comprises relation of detectable signal to the presence or amount of said single troponin form in said sample.

4. The immunoassay system of claim 1 wherein the conjugate antibody of step a) is an insensitive antibody and specifically binds at least two troponin forms; and step d) comprises relation of detectable signal to the presence or amount of a group of troponin forms in said sample.

5. The immunoassay system of claim 1 wherein the capture antibody of step c) is an insensitive antibody and specifically binds at least two troponin forms; and step d) comprises relation of detectable signal to the presence or amount of a group of troponin forms in said sample.

6. The immunoassay system of claim 1 for
determining the presence or amount of troponin I in a whole
blood, plasma or serum sample suspected of containing
troponin complexes or components from damaged heart muscle,
said system comprising:

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a) formation of an antibody conjugate comprising an
antibody coupled to a signal generating element,
said antibody capable of binding to cardiac
specific regions of troponin I complexes and to
unbound troponin I;

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b) formation of a reaction mixture comprising said
whole blood, plasma or serum sample incubated with
said antibody conjugate;

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c) application of said reaction mixture to a solid
phase to which is bound at least one capture
antibody complementary to the antibody conjugate,
said capture antibody binding said antibody
conjugate, whereby the immobilized conjugate
produces a detectable signal upon formation of
sandwich complexes between troponin I complexes or
unbound troponin I; and,

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d) relation of detectable signal to the presence or
amount of troponin I in said sample.

7. The immunoassay system of claim 1 for determining the presence or amount of troponin I in a whole blood, plasma or serum sample suspected of containing troponin complexes or components from damaged heart muscle, said system comprising:

a) formation of an antibody conjugate comprising an antibody coupled to a signal generating element, said antibody capable of binding to the oxidized form of troponin I or to the reduced form of troponin I;

b) formation of a reaction mixture comprising said whole blood, plasma or serum sample incubated with said antibody conjugate;

c) application of said reaction mixture to a solid phase to which is bound at least one capture antibody complementary to the antibody conjugate, said capture antibody binding said antibody conjugate, whereby the immobilized conjugate produces a detectable signal upon formation of sandwich complexes between troponin I complexes or unbound troponin I; and,

d) relation of detectable signal to the presence or amount of troponin I in said sample.

8. The immunoassay of claim 7 wherein the antibody of step a) is capable of binding to the oxidized form of troponin I and to the reduced form of troponin I.

9. The immunoassay of claim 7 wherein the antibody of step a) is capable of binding to the oxidized form of troponin I or to the reduced form of troponin I where said troponin I is unbound or in a complex.

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10. The immunoassay system of claim 1 for
determining the presence or amount of troponin T in a whole
blood, plasma or serum sample suspected of containing
troponin complexes or components from damaged heart muscle,
said system comprising:

a) formation of an antibody conjugate comprising an
antibody coupled to a signal generating element,
said antibody capable of binding to cardiac
specific regions of troponin T complexes and to
unbound troponin T;

b) formation of a reaction mixture comprising said
whole blood, plasma or serum sample incubated with
said antibody conjugate;

c) application of said reaction mixture to a solid
phase to which is bound at least one capture
antibody complementary to the antibody conjugate,
said capture antibody binding said antibody
conjugate, whereby the immobilized conjugate
produces a detectable signal upon formation of
sandwich complexes between troponin T complexes or
unbound troponin T; and,

d) relation of detectable signal to the presence or
amount of troponin T in said sample.

11. The immunoassay system of claim 1 for determining the presence or amount of bound and unbound troponin T in a whole blood, plasma or serum sample suspected of containing troponin complexes or components from damaged heart muscle, said system comprising:

a) formation of an antibody conjugate composition comprising at least two antibodies each coupled to a signal generating element, at least one of said antibodies capable of binding to the cardiac specific regions of troponin T complexes and at least one of said antibodies capable of binding to unbound troponin T;

b) formation of a reaction mixture comprising said whole blood, plasma or serum sample incubated with said antibody conjugate;

c) application of said reaction mixture to a solid phase to which is bound at least one capture antibody complementary to each antibody of the antibody conjugate, said capture antibody binding the bound troponin:antibody conjugate complex to which it is complementary, whereby the immobilized conjugate produces a detectable signal upon formation of sandwich complexes between troponin T complexes or unbound troponin T; and,

d) relation of detectable signal to the presence or amount of troponin T in said sample.

12. The immunoassay system of claim 1 for determining the presence or amount of bound and unbound troponin I in a whole blood, plasma or serum sample suspected of containing troponin complexes or components from damaged heart muscle, said system comprising:

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a) formation of an antibody conjugate composition comprising at least two antibodies each coupled to a signal generating element, at least one of said antibodies capable of binding to the cardiac specific regions of troponin I complexes and at least one of said antibodies capable of binding to unbound troponin I;

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b) formation of a reaction mixture comprising said whole blood, plasma or serum sample incubated with said antibody conjugate;

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c) application of said reaction mixture to a solid phase to which is bound at least one capture antibody complementary to each antibody of the antibody conjugate, said capture antibody binding the bound troponin:antibody conjugate complex to which it is complementary, whereby the immobilized conjugate produces a detectable signal upon formation of sandwich complexes between troponin I complexes or unbound troponin I; and,

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d) relation of detectable signal to the presence or amount of troponin I in said sample.

13. The immunoassay system of claim 1 for determining the presence or amount of troponin I in a whole blood, plasma or serum sample suspected of containing unbound troponin I, troponin I binary complexes, and ternary complexes, said system comprising:

a) formation of an antibody conjugate composition comprising at least two antibodies each coupled to a signal generating element, at least one of said antibodies capable of binding to the troponin T component of the ternary complexes and at least one of said antibodies capable of binding to unbound troponin I and to binary troponin I molecules, wherein said antibody conjugate binds to ternary complexes through the troponin T specific antibody and binds unbound troponin I and binary troponin I molecules through the troponin I specific antibody;

b) formation of a reaction mixture comprising said sample incubated with said antibody conjugate;

c) application of said reaction mixture to a solid phase to which is bound in one zone at least one capture antibody complementary to each antibody of the antibody conjugate, said capture antibody binding the bound troponin:antibody conjugate complex to which it is complementary, whereby the immobilized conjugate produces a detectable signal upon binding unbound troponin I or troponin complexes containing troponin I; and,

d) relation of detectable signal to the presence or amount of troponin I in said sample.

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14. The immunoassay system of claim 1 for determining the presence or amount of troponin T in a whole blood, plasma or serum sample suspected of containing unbound troponin T, troponin T binary complexes, and ternary complexes, said system comprising:

a) formation of an antibody conjugate composition comprising at least two antibodies each coupled to a signal generating element, at least one of said antibodies capable of binding to the troponin I component of the ternary complexes and at least one of said antibodies capable of binding to unbound troponin T and to binary troponin T molecules, wherein said antibody conjugate binds to ternary complexes through the troponin I specific antibody and binds unbound troponin T and binary troponin T molecules through the troponin T specific antibody;

b) formation of a reaction mixture comprising said sample incubated with said antibody conjugate;

c) application of said reaction mixture to a solid phase to which is bound in one zone at least one capture antibody complementary to each antibody of the antibody conjugate, said capture antibody binding the bound troponin:antibody conjugate complex to which it is complementary, whereby the immobilized conjugate produces a detectable signal upon binding unbound troponin T or troponin complexes containing troponin T; and,

d) relation of detectable signal to the presence or amount of troponin T in said sample.

15. The immunoassay system of claim 1 for determining the presence or amount, in a distinct zone or zones, of troponin I in a whole blood, plasma or serum sample, said system comprising:

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a) formation of an antibody conjugate comprising an antibody coupled to a signal generating element, said antibody capable of binding to troponin I when bound in troponin complexes and to unbound troponin I;

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b) formation of a reaction mixture comprising said serum sample incubated with said antibody conjugate;

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c) application of said reaction mixture to a solid phase having at least one discrete zone, said zone comprising at least one immobilized capture antibody capable of binding complexes of troponin I or unbound troponin I; whereby a detectable signal is generated upon the formation of a sandwich complex in said discrete zone; and,

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d) relation of detectable signal to the presence or amount of troponin I in said zone.

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16. The system of claim 15 wherein step c) comprises application of said reaction mixture to a solid phase having at least two discrete zones, said first zone comprising at least one immobilized capture antibody capable of binding complexes of troponin I, and said second zone comprising at least one immobilized capture antibody capable of binding unbound troponin I, whereby a detectable signal is generated upon the formation of a sandwich complex in any one of said two zones; and, step d) comprises relation of detectable signal to the presence or amount of troponin I in said zones, whereby the total amount of troponin in said zones corresponds to the total amount of troponin in said sample.

17. The system of claim 15 wherein step c) comprises application of said reaction mixture to a solid phase having at least three discrete zones, said first zone comprising at least one immobilized capture antibody capable of binding ternary complex, said second zone comprising at least one immobilized capture antibody capable of binding binary complexes of troponin I, and said third zone comprising at least one immobilized capture antibody capable of binding unbound troponin I, whereby a detectable signal is generated upon the formation of a sandwich complex in any one of said three zones; and, step d) comprises relation of detectable signal to the presence or amount of troponin I in said zones, whereby the total amount of troponin in said zones corresponds to the total amount of troponin in said sample.

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19. The immunoassay system of claim 1 for determining the presence or amount, in a distinct zone or zones, of troponin T in a whole blood, plasma or serum sample, said system comprising:

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a) formation of an antibody conjugate comprising an antibody coupled to a signal generating element, said antibody capable of binding to troponin T when bound in troponin complexes and to unbound troponin T;

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b) formation of a reaction mixture comprising said sample incubated with said antibody conjugate;

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c) application of said reaction mixture to a solid phase having at least one discrete zone, said zone comprising at least one immobilized capture antibody capable of binding complexes of troponin T or unbound troponin T; wherein a detectable signal is generated upon the formation of a sandwich complex in said discrete zone; and,

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d) relation of detectable signal to the presence or amount of troponin T in said zone.

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20. The system of claim 19 wherein step c) comprises application of said reaction mixture to a solid phase having at least two discrete zones, said first zone comprising at least one immobilized capture antibody capable of binding complexes of troponin T, and said second zone comprising at least one immobilized capture antibody capable of binding unbound troponin T, whereby a detectable signal is generated upon the formation of a sandwich complex in any one of said two zones; and, step d) comprises relation of detectable signal to the presence or amount of troponin I in said zones, whereby the total amount of troponin in said zones corresponds to the total amount of troponin in said sample.

21. The system of claim 20 wherein said capture antibodies are specific to the interfaces of the binding domains of troponin T/I and T/C.

22. The system of claim 19 wherein step c) comprises application of said reaction mixture to a solid phase having at least three discrete zones, said first zone comprising at least one immobilized capture antibody capable of binding ternary complex, said second zone comprising at least one immobilized capture antibody capable of binding binary complexes of troponin T, and said third zone comprising at least one immobilized capture antibody capable of binding unbound troponin T, whereby a detectable signal is generated upon the formation of a sandwich complex in any one of said three zones; and, step d) comprises relation of detectable signal to the presence or amount of troponin I in said zones, whereby the total amount of troponin in said zones corresponds to the total amount of troponin in said sample.

23. The system of claim 1 wherein a troponin inhibitor is added to said sample prior to formation of said reaction mixture so that said assay system predominantly measures unbound troponin.

24. The system of claim 23 wherein said inhibitor is selected from the group consisting of metal chelates, mastoparan and melittin.

25. The system of claim 1 wherein troponin C is added to said sample prior to the formation of said reaction mixture so that all or substantially all of troponin I or T being measured in said sample will be bound by the troponin C during the course of the assay.

26. The system of claim 1 wherein troponin C and T are added to said sample prior to the formation of said reaction mixture so that all or substantially all of troponin I in the form of the ternary troponin complex is bound during the course of the assay.

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27. The system of claim 1 wherein troponin C and I are added to said sample prior to the formation of said reaction mixture so that all or substantially all of troponin T in the form of the ternary troponin complex is bound during the course of the assay.

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28. The system of claim 1 wherein said antibody conjugate comprises a cocktail of antibodies each coupled to a signal generating element and where a cocktail of capture antibodies are bound to said surface.

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29. The immunoassay system of claim 1 for determining the presence or amount of troponin components in a whole blood, plasma or serum sample suspected of containing troponin from damaged heart muscle, said system comprising:

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a) formation of an antibody conjugate comprising an antibody coupled to a signal generating element, said antibody being one that yields an assay response that is insensitive with regard to the form of troponin I, said antibody being capable of binding to cardiac specific regions of troponin I complexes and to unbound troponin I;

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b) formation of a reaction mixture comprising said sample incubated with said antibody conjugate;

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c) application of said reaction mixture to a solid phase to which is bound at least one capture antibody complementary to the antibody conjugate, said capture antibody binding said antibody conjugate, whereby the immobilized conjugate produces a detectable signal upon formation of sandwich complexes between troponin I complexes or unbound troponin I; and,

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d) relation of detectable signal to the presence or amount of troponin I in said sample.

30. The immunoassay system of claim 29 wherein
step a) comprises forming a conjugate with an antibody
yielding an assay response that is insensitive with regard to
the unbound oxidized and unbound reduced forms of troponin I
and ternary complex; and, step d) comprises relation of
detectable signal to the presence or amount of unbound
oxidized and unbound reduced troponin I and ternary complex
in said sample.

31. The immunoassay system of claim 30 wherein
step a) comprises forming a conjugate with an antibody
yielding an assay response that is insensitive with regard to
the unbound oxidized and unbound reduced forms of troponin I;
and, step d) comprises relation of detectable signal to the
presence or amount of unbound oxidized and unbound reduced
troponin I in said sample.

32. The immunoassay system of claim 30 wherein
step a) comprises forming a conjugate with an antibody
yielding an assay response that is insensitive with regard to
the unbound oxidized form of troponin I and ternary complex;
and, step d) comprises relation of detectable signal to the
presence or amount of unbound oxidized troponin I and ternary
complex in said sample.

33. The immunoassay system of claim 1 for determining the presence or amount of troponin components in a whole blood, plasma or serum sample suspected of containing troponin from damaged heart muscle, said system comprising:

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a) formation of an antibody conjugate comprising an antibody coupled to a signal generating element, said antibody being one that yields an assay response that is insensitive with regard to the complexed or unbound forms of troponin T;

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b) formation of a reaction mixture comprising said sample incubated with said antibody conjugate;

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c) application of said reaction mixture to a solid phase to which is bound at least one capture antibody complementary to the antibody conjugate, said capture antibody binding said antibody conjugate, whereby the immobilized conjugate produces a detectable signal upon formation of sandwich complexes between troponin T complexes or unbound troponin T; and,

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d) relation of detectable signal to the presence or amount of troponin T in said sample.

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34. The immunoassay system of claim 33 wherein
step a) comprises forming a conjugate with an antibody
yielding an assay response that is insensitive with regard to
the unbound form of troponin T and ternary complex; and, step
5 d) comprises relation of detectable signal to the presence or
amount of unbound troponin T and ternary complex in said
sample.

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35. The immunoassay system of claim 1 for determining the presence or amount of troponin components in a whole blood, plasma or serum sample suspected of containing troponin from damaged heart muscle, said system comprising:

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a) formation of an antibody conjugate composition, said composition comprising a cocktail of sensitive antibodies, whereby said cocktail yields an assay response that is insensitive with regard to the forms of troponin I, said composition comprising antibodies capable of binding to cardiac specific regions of troponin I complexes and to unbound troponin I;

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b) formation of a reaction mixture comprising said sample incubated with said antibody conjugate composition;

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c) application of said reaction mixture to a solid phase to which is bound at least one capture antibody complementary to each antibody of the antibody conjugate, said capture antibody binding the bound troponin:antibody conjugate complex to which it is complementary, whereby the immobilized conjugate produces a detectable signal upon formation of sandwich complexes between troponin I complexes or unbound troponin I; and,

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d) relation of detectable signal to the presence or amount of troponin I in said sample.

36. The immunoassay system of claim 35 wherein
step a) comprises forming a conjugate with an antibody
yielding an assay response that is insensitive with regard to
the unbound oxidized and unbound reduced forms of troponin I
and ternary complex; and, step d) comprises relation of
detectable signal to the presence or amount of oxidized and
reduced troponin I in said sample.

37. The immunoassay system of claim 36 wherein
step a) comprises forming a conjugate with an antibody
yielding an assay response that is insensitive with regard to
the unbound oxidized and unbound reduced forms of troponin I;
and, step d) comprises relation of detectable signal to the
presence or amount of oxidized and reduced troponin I in said
sample.

38. The immunoassay system of claim 36 wherein
step a) comprises forming a conjugate with an antibody
yielding an assay response that is insensitive with regard to
the unbound oxidized form of troponin I and ternary complex;
and, step d) comprises relation of detectable signal to the
presence or amount of unbound oxidized troponin I and ternary
complex in said sample.

39. The immunoassay system of claim 1 for determining the presence or amount of troponin components in a whole blood, plasma or serum sample suspected of containing troponin from damaged heart muscle, said system comprising:

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a) formation of an antibody conjugate composition, said composition comprising a cocktail of sensitive antibodies, whereby said cocktail yields an assay response that is insensitive with regard to the unbound or complexed forms of troponin T;

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b) formation of a reaction mixture comprising said sample incubated with said antibody conjugate composition;

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c) application of said reaction mixture to a solid phase to which is bound at least one capture antibody complementary to each antibody of the antibody conjugate, said capture antibody binding the bound troponin:antibody conjugate complex to which it is complementary, whereby the immobilized conjugate produces a detectable signal upon formation of sandwich complexes between troponin T complexes or unbound troponin T; and,

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d) relation of detectable signal to the presence or amount of troponin T in said sample.

40. The immunoassay system of claim 39 wherein
step a) comprises forming a conjugate with an antibody
yielding an assay response that is insensitive with regard to
unbound form of troponin T and ternary complex; and, step d)
comprises relation of detectable signal to the presence or
amount of unbound troponin T and ternary complex in said
sample.

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41. The immunoassay system of claim 1 for independently determining the presence or amount of: 1) the cardiac ternary complex; 2) the cardiac troponin binary complex of I(oxidized)/T; 3) the cardiac troponin binary complex of I(reduced)/T; 4) the cardiac troponin binary complex of I(oxidized)/C; 5) the cardiac troponin binary complex of I(reduced)/C; 6) the cardiac troponin binary complex T/C; 7) unbound cardiac troponin I (oxidized); 8) unbound cardiac troponin I (reduced) and 9) unbound cardiac troponin T, in a whole blood, plasma or serum sample suspected of containing troponin from damaged heart muscle, said system capable of determining the presence of n forms of troponin, where n is a whole integer of from 1 to 9, said system comprising:

a) formation of an antibody conjugate composition comprising an antibody that binds troponin, wherein said antibody is coupled to a signal generating element;

b) formation of a reaction mixture comprising said sample incubated with said antibody conjugate;

c) application of said reaction mixture to a solid phase having n discrete zones, wherein the discrete zone comprises:

1) a zone comprising an immobilized capture antibody capable of binding said cardiac ternary complex;

2) a zone comprising an immobilized capture antibody capable of binding said cardiac troponin binary complex I (oxidized)/T;

3) a zone comprising an immobilized capture antibody capable of binding said cardiac troponin binary complex I(reduced)/T;

5 4) the cardiac troponin binary complex of I(oxidized)/C;

5) the cardiac troponin binary complex of I(reduced)/C; 6) a zone comprising an immobilized capture antibody capable of binding said cardiac troponin binary complex T/C; 7) a zone comprising
10 an immobilized capture antibody capable of binding said unbound cardiac troponin I (oxidized);

8) a zone comprising an immobilized capture antibody capable of binding said unbound cardiac troponin I (reduced); or,

15 9) a zone comprising an immobilized capture antibody capable of binding said unbound cardiac troponin T, whereby a detectable signal is generated upon the formation of a sandwich complex in said discrete zone; and,

20 d) relation of detectable signal to the presence or amount of n forms of troponin from damaged heart muscle.

25 42. The immunoassay system of claim 41 wherein step a) further comprises that the antibody that binds troponin is an antibody that yields an assay response that is insensitive with regard to the form of troponin.

5 43. The immunoassay system of claim 42 wherein
step c) further comprises that the immobilized capture
antibody of said discrete zone is an antibody that yields an
assay response which is sensitive with regard to the form of
troponin.

10 44. The immunoassay system of claim 41 wherein
step a) further comprises that the antibody that binds
troponin is an antibody that yields an assay response that is
sensitive with regard to the form of troponin.

15 45. The immunoassay system of claim 44 wherein
step c) further comprises that the immobilized capture
antibody of said discrete zone is an antibody that yields an
assay response which is insensitive with regard to the form
of troponin.

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d) relation of detectable signal to the presence or amount of troponin C in said sample.

47. A stabilized composition of troponin.

5 48. The stabilized composition of claim 47
comprising a stabilized composition of troponin I, wherein
the troponin I is oxidized.

49. The stabilized composition of claim 48 wherein
the troponin I is unbound or the troponin I is in a complex.

10 50. The stabilized composition of claim 49
comprising a stabilized composition of the ternary complex of
troponin I, T and C.

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51. A method for improving the recovery of troponin I or T from a surface used in immunoassays, said method comprising: contacting with said surface at least one strongly basic peptide, protein, or polymer with a pI value greater than about 8.

52. The method of claim 51, further comprising a step of washing unbound peptide, protein or polymer from said surface.

53. The method of claim 51 wherein melittin is the strongly basic peptide used.

54. The method of claim 51 wherein protamine is the strongly basic protein used.

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